

Application No. 10/538,423

Paper Dated: August 25, 2008

In Reply to USPTO Correspondence of February 25, 2008

Attorney Docket No. 4544-051674

**AMENDMENTS TO THE SPECIFICATION**

Please replace the first paragraph after BACKGROUND OF THE INVENTION on page 1 with the following rewritten paragraph:

-- In agricultural biotechnology a long standing goal is to improve tolerance of crop plants to environmental stress such as salinity, drought and temperature mediated dehydration all of which constitute direct osmotic stress. ~~OneeOne~~ of the mechanisms by which plants respond to such abiotic stress conditions is by synthesizing non-toxic biomolecules termed compatible solutes or osmoprotectants. These compounds fall into three categories; amino acids (eg proline), onium compounds (eg glycinebetaine, dimethylsulphoniopropionate) and polyols/sugars (eg inositol, ononitol/pinitol mannitol, trehalose). Over production of any such osmoprotectant by introgression of genes encoding critical steps in the synthesis of these compounds through metabolic engineering has become the choice of biotechnologists for raising stress tolerant crop plants. Such approaches have met limited success in both ~~pre~~ prokaryotic and eukaryotic systems. More importantly, it is imperative that the critical step for manipulation should itself encode a stress-tolerant enzyme protein. --

Please replace the first paragraph after BRIEF DESCRIPTION OF THE INVENTION on page 2 with the following rewritten paragraph:

-- The present invention, provides a salt-tolerant L-myo-inositol +1-phosphate synthase from *Porteresia coarctata*. --

Please replace the first paragraph after DETAILED DESCRIPTION OF THE INVENTION on page 3 with the following rewritten paragraph:

-- Cloning and sequencing of L-myo-inositol 1-phosphate synthase ~~gene~~ from *Porteresia coarctata* (PINO1) and its comparison with that from *Oryza saliva* (RINO1). --

Please replace paragraph 2. on page 4 with the following rewritten paragraph:

-- 2. Second round with primers designed at the 5' end and the 3' end of the ~~gene~~CDNA-as used for RT-PCR amplification. --

Please replace the first full paragraph on page 7 with the following rewritten paragraph:

-- On analysis it was revealed that the nucleotide sequences of the PINO1 ~~gene~~ is considerably non-identical resulting in ~~gene~~-products in which the RINO1 and PINO1 differ in

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the amino acid sequences for a stretch of about 110 in the mid-portion (between amino acids 173 to amino acids 320 of PINO1), the other parts of the genessequences bearing complete identity. The non-identical portion ~~compriseis comprised~~ of deletions/additions as well as conservative substitutions with two additional amino acids in case of PINO1 resulting in a protein having 512 amino acids ~~instead~~instead of reported 510 amino acids of RINO1. --

Please replace the second full paragraph beginning on page 7 with the following rewritten paragraph:

-- The cDNA for RINO1 and PINO1 were subcloned into suitable cloning sites of the bacterial expression vector pET 20B (+). The resulting plasmids were introduced into the host strain E. coli BL-21 (DE3). The bacteria were grown in LB medium up to A<sub>600</sub> of 0.5-absorbance unit and induced by 0.5 mM IPTG for 6 hours at 30°C. The bacteria were collected by centrifugation and lysed by sonication in a buffer containing 20mM Tris-HCl, pH 7.5, 10 mM each of NH<sub>4</sub>Cl and ME, and 2mM PMSF. The lysed extracts were centrifuged and protein from both soluble and membrane fractions were analyzed by 10% SDS-PAGE according to Laemmli (Nature, 227, 680-685, 1970) followed by western blot for immunodetection. The separated proteins were blotted onto PVDF membrane and the blot was probed with rabbit anti L-myo-inositol 1-phosphate synthase antibody (1:500) raised against purified recombinant L-myo-inositol 1-phosphate synthase of *Entamoeba* (Lohia et al, Mol.Biochem.Parasitol., 98, 67-79,1999) or purified cytosolic L-myo-inositol 1-phosphate synthase from Oryza leaves. Bound antibody was detected by the chemiluminescence (kit from Amersham Life Sciences). Results of such experiments indicated that both RINO1 and PINO1 were expressed predominantly in the membrane fractions (Fig 2, A & B, lanes 1 & 3). --